BABA GHULAM SHAH BADSHSH UNIVERSITY
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SYNOPSIS FOR Ph.D REGISTRATION

Proposed Research Topic	**:**	Studies on taxonomy of nematodes of the order Mononchida in Jammu division of Jammu and Kashmir State, India
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Subject	**:**	Zoology
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Background and Justification

Surpassing all the organisms not only in number but in their species diversity also nematodes, which are popularly known as roundworms, are the most numerous organisms on earth. Four of every five multicellular animals on the planet are nematodes. Perhaps it has quite rightly been said that nearly ninety percent of all the metazoa in the world are nematodes. They are placed at a very low level of taxonomic hierarchy in the animal kingdom in the company of Rotifera, Gastrotricha, Kinorhyncha and Nematomorpha. The distribution of these organisms ranges from deep ocean trenches to the top of high mountains encompassing all possible habitats including hot springs, ice, low oxygen, acid environments, etc. Some of them can withstand complete dryness on the surface of rocks. Their size too is extremely variable ranging from less than 100 μm (*Greefiella minutum*) to greater than 8 m (*Placentonema gigantissma*). Their abundance reaches many million individuals per m^3 in soil and bottom sediments of aquatic habitats and a handful of soil may contain more than 50 species. Some species of nematodes may contain more than 27 million eggs at one time and lay more than 200,000 of them in a single day.

Knowledge of animal parasitic nematodes is very ancient and dates back to about 1,500 B.C., when the dreaded Guinea worm, *Dracunculus medinensis* and the large round worm, *Ascaris lumbricoides* were known. However, marine, freshwater, soil and plant parasitic nematodes remained little known groups mainly because of their extremely small size and the difficulties encountered in their isolation, mounting and observation. Borellus (1656) for the first time observed the vinegar eel worm, *Turbatrix aceti* and Needham (1743) was the first to observe a plant parasitic nematode, *Anguina tritici* in the cockles of wheat when he examined a portion of the crushed cockle under his primitive microscope. Among the soil inhibiting organisms, the nematodes dominate over all the others both in numbers and species. These

nematodes may be placed under five categories (Yeates, 1998. Yeates & Bongers, 1999), Plant feeders / Herbivore which feed on vascular plants; Hyphal feeders / Fungivores which feed on fungal hyphae by a stylet with narrow lumen; Bacterial feeders / bactrivores which feed on a range of food source; Omnivores which feeds on bacteria and fungus and Predators which feed on nematodes or other soil micro-organisms.

Mononchs are of great agricultural and economical importance by acting as bio-control agent against phytoparasitic nematodes and other soil microorganisms as suggested by Cobb (1917). Thorne (1927) further emphasized the use of mononchs in bio-control and stated that a large population of *Clarkus papillatus* could be useful in the control of *Heterodera radicicola*. Dujardin (1845) reported first predatory nematode *Oncholaimus muscorum, O. fovearum* and *Enoplus crassiculus*. Bastian (1865) proposed the genus *Mononchus* and also described five new species *viz., M. truncatus, M. papillatus, M. macrostoma, M. tumbridgensis* and *M. cristatus* and transferred three species *Oncholaimus muscorum, O. fovearum* and *Enoplus crassiculus* under the genus *Mononchus*. In the next 50 years (1865-1915) only 20 more species were added to this group. Cobb (1916) provided details on the systematics, morphology, biology and ecology of mononchs. He split the genus *Mononchus* into five subgenera *Mononchus, Prionchulus, Mylonchulus, Iotonchus* and *Anatonchus* and later Cobb (1917) proposed the subgenus *Sporonchulus* and described 32 new species. Micoletzky (1922) recognized 41 species of mononchs. Chitwood (1937), proposed the family Mononchidae under the superfamily Tripyloidea, of suborder Enoplina, of the order Enoplida for the genera *Mononchus* and *Prionchulus* and subgenera *Mylonchulus, Iotonchus, Anatonchus* and *Sporonchulus*. Later full generic status was given to *Anatonchus* by de Coninck (1939) *Iotonchus* and *Mylonchulus* by Altherr (1950; 1953 respectively) and *Sporonchulus* by Pennak (1953). Andrássy (1958)

provided a valuable revision of the mononchs and added five more genera to the family Mononchidae, *viz*., *Brachonchulus, Cobbonchus, Granonchulus, Judonchulus* and *Miconchus*. Clark (1961) raised Mononchidae to superfamily status Mononchoidea and shifted it from suborder Enoplina to Dorylaimina. He (l.c.) also proposed the family Bathyodontidae for genus *Bathyodontus*, *Oionchus* and *Mononchulus* under Mononchoidea. Provided information on evolution and correlated the shape of buccal cavity with pharyngeo-intestinal junction. Mulvey (1961a, b; 1962, 1963a, b & 1967a, b) in a series of publications described a good number of new species belonging to various genera of mononchs. He redescribed and revised several known species and also provided the keys for their identification. Mulvey & Jensen (1967) proposed four new genera *viz*., *Crassibucca*, *Hadronchus, Polyonchulus* and *Prionchulellus,* and described 16 new species. Coetzee (1965, 1966, 1967a, b & 1968) described several new and known species of the genera *Cobbonchus*, *Granonchulus*, *Mylonchulus, Iotonchus*, *Mononchus* and *Prionchulus.* Jairajpuri contributed a lot on the systematics of mononchs. He (1969, 1970a, b & 1971) described Indian species of the genera *Hadronchus*, *Miconchus*, *Mononchus*, *Iotonchus*, *Clarkus*, *Sporonchulus*. He (1969) separated mononchs from Dorylaimida and gave it an independent ordinal rank. Further, in 1971 he recognized two suborders: Mononchina Kirjanova and Krall, 1969 with superfamilies Mononchoidea and Anatonchoidea Jairajpuri, 1969 and Bathyodontina Coomans & Loof, 1970 with superfamilies Bathyodontoidea, Clark, 1961 and Mononchuloidea, de Coninck, 1965. Jairajpuri and Khan (1982) provided detailed information on the morphology and systematics of the mononchs of India gave a revised classification of the group and provided keys to higher taxa and species.Different authorities have different opinions regarding the taxonomic status of mononchs. Mononchida Jairajpuri (1969) proposed as a separate order for this group has been accepted by majority of workers. However, a few

nematologists still consider mononchs and bathyodonts only at subordinal rank, under the order Dorylaimida. On the other hand, Mulvey (1978) regarded mononchs still to represent just a family - Mononchidae *sensu* Chitwood, 1937.Mononchs and dorylaims are related in their body organization, similar type of oesophageal gland nuclei and orifices and in having almost similar type of reproductive organs. However, they have marked differences in their stoma, pharynx, pharyngeo-intestinal junction, lateral guiding pieces / gubernaculum, caudal glands, spinneret, absence of pre-rectum and pre-anal pair of supplements and feeding habit. Based on the above marked differences mononchs and dorylaims are kept under separate orders. Originally the genus *Mononchus* Bastian, 1865 was a species complex and contained over 80 described species Williams (1958) which include many of the commonest free-living nematodes inhabiting soil and fresh water. It is of world-wide distribution and several species are cosmopolitan. All members of the genus appear to be obligate predators upon the microfauna of their habitat.

Jairajpuri (1970) splited *Mononchus* into two distinct groups, the first group represented by type species *M. truncatus* Bastian, 1865. It contains species which generally have lips and papillae reduced, buccal cavity elongate cylindroid, tail elongate with smoothly rounded terminus, spicules and accessory pieces strikingly long and slender and caudal glands and spinneret well developed. Species of this group are either aquatic or show a distinct preference for wet soils. The second group, represented by *M. papillatus* Bastian, 1865, contains species which generally have lips and papillae well developed, buccal cavity barrel shaped, tail short conoid and arcuate, spicules and accessory pieces short and stout, caudal glands absent or rudimentary and spinneret absent. Species of this group do not show any preference for wet soils. This group is much closer to the genus *Prionchulus* Cobb (1916), Wu and Hoeppli (1929). Based on the above characters he splitted the genus *Mononchus* into two distinct genera: i)

Mononchus, Bastian (1865) retaining only the species of *M. truncatus* group, ii) *Clarkus*, Jairajpuri, 1970: containing all other species belonging to *M. papillatus* group. This division of *Mononchus sensu lato* is in conformity with the views expressed by Clark (1962). The genera *Prionchulus* Cobb (1916), Wu and Hoeppli (1929) *Clarkus* Jairajpuri, (1970).

The worthwhile studies on taxonomy of Mononchs in India started in 1969's and over the years large number of taxa have been described from various parts of country. As regards of taxonomic work on Mononchs nematode fauna of Jammu and Kashmir are scanty, as only three species of Mononchs are reported from the region.(*Mononchus tunbridgensis*, *Prionchulus muscorum, Anatonchus kashmirensis*) To fill this gap the present plan of work is proposed to study the taxonomy of nematodes belonging to order Mononchida in Jammu division of Jammu and KashmirState, India. The study area for the proposed research plan will involves following areas.

1. Poonch district (Dera ki Gali, Peer ki Gali)
2. Samba district (Mannsar lake)
3. Ramban district (Patnitop, Baghliar)
4. Jammu district (Akhnoor, chokichora, Kalidar)
5. Reasi district (Mohoor)
6. Rajouri district (Budhal, Darhal, Thannamandi, and upper reaches of Ghambir Mughlan)
7. Doda district (Bhadarwah)
8. Kishtiwar district (Kishtiwar)
9. Kahua district (Bani)
10. Udhampur district (Ramnagar)

Objectives of the study:

- Survey, collection, and identification of Mononchid nematodes in Jammu division of Jammu and Kashmir State.
- Description of all the Mononchid taxa isolated**.**
- Molecular characterization of closely related taxa using appropriate markers.

Methodology

(a) Methodology for Taxonomic Study:

Classical Taxonomy

For morphological identification the methodology involves the following steps:-

Collection of samples: Samples for the study of Mononchid nematodes will be collected from different parts of Jammu division of Jammu and Kashmir State, India. The samples will be collected from different types of habitats like forests, lakes, canals etc in different seasons. Most samples will be collected from undisturbed soils. The samples will be kept in air tight polythene bags. All relevant information's such as locality, date of collection, etc. will be noted. The samples will be brought to the laboratory for further processing.

Processing of samples: To extract the nematodes the soil will be processed by the modified Cobb's (1918) sieving and decantation and modified Baermann's funnel techniques. The sample will be placed in a bucket and thoroughly mixed in a small amount of water to break the soil crumbs manually. Stones and debris will be removed by hand and the bucket will then be filled with water. This suspension will be stirred gently to make it homogeneous and will be left undisturbed for about 30 sec. to allow the heavy particles to settle down at the bottom. The

suspension will then be poured into another bucket through a coarse sieve (2mm pore size) which retained debris and leaves. The suspension in the second bucket will then be poured through a 300 mesh sieve (53µm pore size). The nematodes and fine residue will retained on the sieve. The process will be repeated thrice for better recovery of nematodes and the residues will be collected in a beaker.

Isolation of nematodes: Nematodes will be isolated by using modified Cobb's sieving and decantation and modified Baermann's funnel technique.

Killing and fixation of nematodes: The nematodes will be killed and fixed in hot FA 4:1 (Seinhorst, 1962).

Clearing, mounting and sealing: After fixation the nematodes will be transferred into a mixture of glycerine-alcohol (5 parts glycerine + 95 parts 30% alcohol). Gradual dehydration will be done by using anhydrous calcium chloride. Mounting will be done in anhydrous glycerine and subsequent sealing by wax.

Morphometric and Allometric analysis: The measurements will be taken by using an ocular micrometer and dimensions will be expressed by using De Man's formula. The illustrations will be drawn with the help of a drawing tube attached to Olympus microscopes.

Photography: Photographs will be taken by using a digital camera attached to Olympus microscope.

The standard taxonomic procedures will be followed.

Molecular Taxonomy

b) Methodology for molecular taxonomy:

Nematodes will be cultured and maintained in agar medium till their final use. Nematode extraction buffer (NEB) method proposed by Sambrook and Russel (2001) and Hashmi *et al*, **(**1992) with modifications wherever required shall be followed for genomic DNA isolation. After purification, the extracted worm DNA shall be subjected for appropriate marker analysis.

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